Marine-Derived Bioactive Peptides and Their Health-Promoting Properties

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**ABSTRACT**

Bioactive peptides are chains of acids that have the ability to produce specific effects on the human body. These peptides can be obtained from sources, such as animal, plant and microbial proteins. Among these marine derived bioactive peptides possess a significant place in the literature. In years a lot of research has been conducted on producing, purifying and characterizing peptides because of their potential to promote good health. This chapter aims to explore the methods used in generating marine derived peptides and the techniques employed for their purification and characterization. Moreover, it will delve into the range of health benefits associated with consuming bioactive peptides.

**Keywords-** Bioactive peptides, marine peptides, bioactive properties, human health

1. **INTRODUCTION**

The scientific community acknowledges oceans as a major supplier of bioactive compounds. The vast expanse of oceans, which occupy 70% of the Earth’s surface is a great reservoir of bioactive compounds that can be listed as follows: polyunsaturated fatty acids (PUFA), gelatin, collagen, minerals, antioxidants, polysaccharides, peptides. These compounds have structures and hold great promise as potential therapeutic agents.

Researchers pay great attention in investigating the health benefits of bioactive peptides derived from marine resources. These small protein fragments are typically made up of 2 to 20 amino acids with molecular weights (MWs) extending from 500 to 1850 Da. Furthermore, in certain investigations, longer peptides have been observed. For instance, Lunasin, a soy based peptide, consists of 43 amino acid residues. Peptides that are small, in size have a chance of easily passing through the intestinal wall without any obstacles allowing them to effectively demonstrate their biological and functional effects. Bioactive peptides have shown potential in promoting well-being and preventing health issues [1].

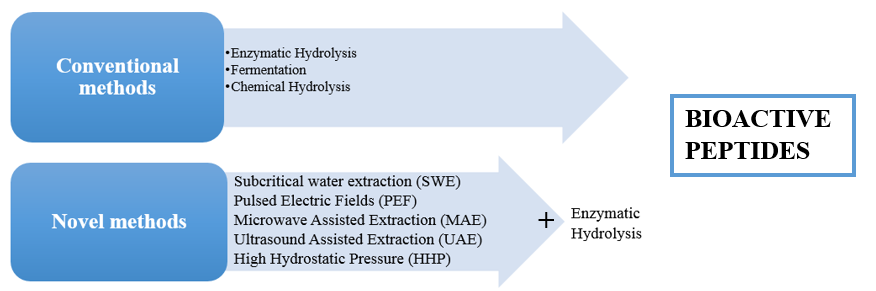
Bioactive peptides obtained from marine resources possess qualities such as antioxidant properties, antimicrobial activity, blood pressure regulation capabilities, anti-diabetic, anti-obesity, anti-inflammatory effects and immune system modulation among others [2]. Bioactive peptides offer benefits over conventional drugs, including their lower toxicity and minimal side effects, in humans. Given their range of functionalities, they are considered candidates for application in industries such as food production, pharmaceuticals and nutraceuticals [3]. The BIOPEP-UWM, which is a database containing extensive data on bioactive peptides, presently comprises 4,699 bioactive peptides that have been identified.

Bioactive peptides, while initially inactive within the structure of a parent protein, exhibit a wide range of biological activities after being destroyed via conventional or unconventional methods. These activities found out by sequence of the peptides and the specific amino acid composition [4].

The crude protein hydrolysate comprises a blend of peptides and unbound amino acids. Obtaining these peptides involves several important steps. Firstly, the protein source should be selected and employed various enzymatic, chemical or microbial techniques to produce the peptides. Then it becomes crucial to purify and refine the desired peptides from a mixture of proteins and other substances. In this purification process innovative technologies, like chromatography, ultrafiltration and electro-dialysis play a role. After the purification process, it is crucial to examine the peptides to determine their safety, stability and biological effects. To accurately understand the structure and functions of these peptides advanced analytical methods like mass spectrometry, bioassays and nuclear magnetic resonance (NMR) spectroscopy are utilized.

1. **PRODUCTION, PURIFICATION AND CHARACTERIZATION OF BIOACTIVE PEPTIDES**

In recent decades, it is well known that marine protein offers a high resource of bioactive peptides [5]. Recently, numerous researchers have isolated and reported various kinds of bioactive peptides derived from marine organisms [2]. Bioactive peptides can be found encased in food proteins and they are found passively within the structure. This non-active structure should be destroyed via hydrolysis methods for the emergence of bioactive properties [6]. Chemical hydrolysis, enzymatic hydrolysis and fermentation are conventional techniques for the production of bioactive peptides. Due to the nature of enzymes their low yield, high cost and the limited availability of food-grade enzymes, the researchers shifted their attention to searching for alternatives [7]. Several innovative processing techniques have been arising as substitutes for these kinds of conventional techniques in the production of bioactive peptides (Figure 1).

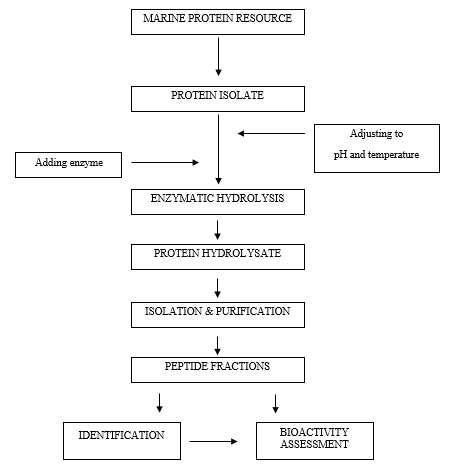


**Figure 1. Main Methods for Production of Bioactive Peptides**

1. **Production**

Chemical hydrolysis techniques are known for their simplicity, speed, and well-established nature, making them a traditional method for peptide production. Protein hydrolysates were obtained using chemical hydrolysis under acidic or alkaline conditions with high temperatures (120°C) and pressure (100 kPa). Alkali methods often yield derivatives of lysinoalanine which is a well-known toxic compound that occurs when essential amino acids like tryptophan or cysteine are hydrolyzed [8]. Additionally, the neutralization phase in chemical hydrolysis methods results in a significant amount of salt generation. Furthermore, chemical techniques are vulnerable to amino acid degradation caused by high temperatures, varying pH levels, and, in certain cases, pressure. Despite the drawbacks such as, lack of specificity, sensitivity, poor nutritional qualities and limited properties, the chemical release of antioxidant peptides is still accepted as more cost-effective in the industrial production of peptides.

The breakdown of protein molecules, through enzymatic hydrolysis is the main method used to produce bioactive peptides (Figure 2). Enzymatic hydrolysis is known for its easily manageable conditions making it a precise method, for breaking peptide bonds. One of its advantages is the absence of side reactions or any loss in value. Moreover, it offers enhanced protein recovery and purification capabilities for peptides. These characteristics have garnered increasing interest in producing hydrolysate with functional and bioactive properties. Usually, the process of hydrolysis occurs in a container called a reactor. In this reactor, various factors such as temperature, pH level, agitation and duration can be controlled. Firstly, pH and temperature had to be adjusted for the protease which is used for hydrolysis. When protease is added, the interaction between the substrate and enzyme leads to alterations in the pH level of solutions as a result of break down of peptide bonds to form carboxyl groups or new amino able to release [9].



**Figure 2. Flow chart of bioactive peptide production by using proteases**

Fermentation, an ancient food preservation method, facilitates the formation of bioactive peptides. Fermented products are typically obtained through both controlled and natural fermentation processes by microorganisms. Lactic acid bacteria play a crucial role in the production of fermented products by contributing essential technological and nutritional functionalities. These properties include texture enhancement and flavor improvement, which are key factors in the fermentation process. The cost of producing peptides through fermentation is comparatively lower than using the enzymatic technique. On the other hand, the industrial utilization of fermentation for peptide production faces obstacles like peptide yield and a lack of specificity, in peptide formation [7].

HHP (High Hydrostatic Pressure) is a non-thermal and eco-friendly technique applied at isostatic pressures between 100 and 1000 MPa. The HHP procedure operates as a batch system where water serves as a medium, for transmitting pressure. Over the past few decades, this processing technique has been extensively studied for its ability to inhibit microbial activity while preserving the organoleptic and nutritional attributes of food products. Therefore, the main benefit of this approach is that it causes minimal interference with biologically active mixtures by employing lower or moderate temperatures [7]. A method called high-pressure processing was used along, with hydrolysis to enhance the breakdown of proteins. High-pressure processing causes proteins to unfold, which makes it easier for enzymes to break them down and speed up the process of protein hydrolysis. To enhance proteolysis a combination of pressure processing and enzymatic hydrolysis (referred to as HHP-assisted hydrolysis) was utilized. The application of pressure causes the protein structure to unfold thereby exposing sites where enzymes can facilitate the hydrolysis of proteins. In the research, the aim was to enhance the characteristics of fish gelatin through the application of hydrostatic pressure (HHP) and ultrasonication (US). The rate at which the pressure was increased was 340 MPa, per minute. This was done until it reached a pressure of 400 MPa. The time taken to release the pressure for each treatment was than 20 seconds. The researchers reported that both the HHP and US processing methods had an impact, on increasing the gel strength of the samples compared to those that were not treated (with a significance of p<0.05). The results from Fourier transform infrared spectroscopy (FTIR) indicated that the treatments caused changes in the conformation of acids. Both treatments yielded improvements in gel characteristics such as gel strength gelling temperature and melting temperature for fish gelatin. Ultimately after assessing parameters it was determined that the optimal combination, for fish gelatin was achieved with a pressurization level of 400 MPa at 10 °C for a duration of 15 minutes [10].

Ultrasonic Assisted Extraction (UAE) is a technology that utilizes ultrasonic waves with frequencies, above 20 kHz to produce peptides. This innovative non-thermal physical technique relies on the transmission of waves through a medium. Acoustic waves, which can be classified based on their similarity to frequencies to humans fall within the range of 20 Hz to 20 kHz. Frequencies below this range are referred to as infrasound whereas those, above it are called ultrasound [11]. Ultrasound has been utilized to address the limitations of enzyme hydrolysis including the conversion rate of protein and lengthy hydrolysis time [12]. The benefits of ultrasound technology such, as enhanced energy and mass transfer, time and temperature requirements, improved process control, selective extraction capabilities and quicker initiation have made it a favored pretreatment method, for generating bioactive peptides. Ultrasound pretreatment has been found to enhance the production of peptides (ranging from 200 to 3000 Da) that are rich, in hydrophobic amino acids and possess antioxidant properties [13].

Water, the liquid, on Earth stands out as a green technology for extracting bioactive compounds when compared to conventional methods that employ organic solvents. Subcritical water (SCW), which possesses toxic and nonflammable properties has gained significant attention due to its ability to extract substances without leaving any solvent residue. Subcritical water refers to water maintained at temperatures betwwen 100°C and 374°C, pressures below the critical point of water (22 MPa). This extraction technique is also called pressurized hot water extraction, pressurized liquid extraction or superheated water extraction. Subcritical water hydrolysis has been widely used to extract molecules like protein hydrolysates in the form of amino acids or peptides. Numerous researchers have utilized water to produce peptides, from plant and animal sources [14]. SCW exhibits the ability to generate hydrolysates, with weight from proteins. When squid muscle was treated with SCW at a temperature of 220°C it resulted in the production of peptides with high antioxidant properties. However, when higher temperatures were used the peptides underwent decomposition. Transformed into organic compounds [15].

A microwave operates by utilizing waves that fall within the range of 300 MHz to 300 GHz. These waves transmit energy through interactions, in the material through processes known as rotation and ionic conduction. Recently scientists have been using microwave assisted (MAE) hydrolysis to extract bioactive peptides from various sources such as rainbow trout frames [16], algae [17], sea cucumber [18]. Some scientists have conducted research on the antioxidant capabilities of sulphated polysaccharides derived from Brown Seaweed. They employed microwave assisted extraction at varying pressures, extraction times and algae/water ratios. The findings of these studies suggested that microwave assisted extraction proved to be a technology. Additionally, mechanical disruption techniques have proven valuable, in breaking down the siliceous skeletons of certain hard sponges [19]. In general, this technique facilitates selective and expedited compound extraction compared to conventional methods. It yields even better results while consuming less energy and solvent volume and it reduces costs. Possesses a more environmentally friendly nature compared to traditional extraction processes [20].

1. **Purification**

Before assessing the biological characteristics of the desired bioactive peptides obtained from marine protein hydrolysate it is essential to purify the peptides. Purification techniques of bioactive peptides involve various methods to isolate and separate the desired bioactive peptides from complex mixtures. These techniques are listed as follows: ultrafiltration, high-performance liquid chromatography (HPLC), gel filtration, reverse-phase chromatography high-performance liquid chromatography (RP-HPLC) and ion change column chromatography. However, there are advantages and disadvantages, to each purification method [21].

Ultrafiltration is a membrane-based method is commonly used to separate peptides of varying weights by utilizing membranes. The most effective fractions can be determined by comparing the ultra-filtration refined hydrolysates with the initial hydrolyzates. Marine protein hydrolysates have been separated into different fractions using filter membranes with various molecular weight cutoffs (MWCO) ranging from 1 to 10 kDa. Furthermore, the purity of ultra-filtered hydrolysates will increase significantly, ranging from 1.51 to 525 times, compared to crude hydrolysates [22].

Ultrafiltration has advantages, including its scalability, independence, from chemical agents and compatibility with other processes. In a study, germinated soybean peptides with DPP IV, α-amylase and α-glucosidase inhibitory activity were separated using ultrafiltration membranes categorized by their weight (<5 kDa, 5–10 kDa and >10 kDa) [27]. Peptides in the kDa and <5 kDa range exhibited inhibitory effects on α-amylase and α-glucosidases respectively. On the other hand, DPP-IV inhibitory peptides demonstrated potency at molecular weights ranging from 5–10 kDa to >10 kDa. Additionally, antimicrobial properties were observed in chia seed hydrolysate derived peptides with weights, below 3 kDa [23].

Gel filtration chromatography (GFC) is known as size exclusion chromatography has been a used technique for more than four decades to separate, desalt and estimate the molecular weight of proteins and peptides. GFC is considered one of the gentlest techniques in chromatography as it separates molecules based on their size differences. The mechanism behind its separation involves filtering molecules according to their sizes; smaller molecules enter the gel pores. Travel a longer distance while larger ones have shorter retention times. Unlike chromatography methods like ion exchange, GFC does not involve molecule binding to the chromatography medium. As a result variations in buffer composition do not directly impact resolution. This provides an advantage for GFC since elution conditions can be adjusted according to sample type and requirements for further purification, analysis or storage without compromising the separation process. GFC is particularly suitable for biomolecules that are sensitive to pH changes, metal ion concentrations or cofactors as well as harsh environmental conditions. It can be employed directly after ion exchange chromatography without affecting the final separation outcome. Moreover, GFC offers selectivity and resolution levels—making it an important step, within purification strategies. There are types of GFC media, each, with unique characteristics. For instance, Superdex Increase or Superdex is specifically created to achieve resolution shorter run times and increased recovery. In a research, conducted a purification process to isolate trypsin inhibitors from the roe of Yellowfin Tuna (*Thunnus Albacores*) [24]. They used column chromatography techniques, Sephacry S 200, Sephadex G 50 and DEAE cellulose. Eventually, they determined that the trypsin inhibitor had a molecular weight of approximately 7 × 104 Da. Furthermore, Sephadex is widely recommended for the separation of different groups in processes such as desalting and buffer exchange. It is extensively utilized in the field of purifying organisms. Although, it can be challenging in terms of time consuming and costly, GFCs remarkable selectivity and high resolution make it useful, for various separation and purification applications [25].

In times, the use of ion exchange chromatography (IEX) techniques has gained significant importance in separating, detecting and determining the structure of proteins, peptides and small nucleotides. IEX media contain groups with charges that attract molecules with an opposite charge. These bound molecules are then released from the medium by introducing a charged molecule with increasing concentration. Proteins have functional groups that can carry positive or negative charges [26]. By adjusting the pH level or ionic concentration of the phase, proteins can be effectively separated. IEX is employed for capturing target proteins or removing bulk impurities from large volumes. It serves as a purification step or, as a final step to achieve high resolution purification by eliminating impurities. Ion exchange, being an adsorption technique offers the flexibility to be employed in both negative capture modes. The choice between these modes depends on the pH or conductivity of the sample. In chromatography the target compound is adsorbed while the contaminant remains unretained. Conversely in chromatography it is the contaminant that gets adsorbed while the target remains unretained. Additionally, there exists an array of ion exchange (IEX) media available, allowing for selecting a suitable medium based on specific requirements such as target compound, sample characteristics and desired resolution. One major benefit of using IEX is its ability to separate masses, which can lead to time savings and improved accuracy compared to GFC. However, it is important to note that IEX can be expensive and complex and may not be suitable for biomolecules that are sensitive to factors such as pH and metal ions. Future research in the field of IEX could focus on discovering a material with high resolution capabilities, which would replace the costly media currently used. While achieving this goal may pose challenges we can expect adoption of IEX, for biological separation in the coming years [25].

1. **Identification**

The investigation of the sequence responsible for the observed bioactivities has not received much attention or has mainly focused on determining the most likely sequence. Initially, peptide identification relied on Edman degradation. However, in recent times, chromatographic techniques, particularly LC-MS/MS methods, have gained more recognition because of their accuracy and sensitivity. These methods are now considered the standard for analyzing bioactive compounds [27].

Mass spectrometry has become a crucial analytical tool in characterizing bioactive molecules, thanks to advancements in ionization techniques and mass analyzers. With these developments, even complex samples can now be effectively analyzed. This includes a wide range of bioactive molecules, from small metabolites to large macromolecular assemblies. This can be attributed to the convergence of advancements that enable the analysis of small quantities of challenging compounds like peptides in complex mixtures along, with the rapid expansion of genomic databases that can be searched using mass spectrometry data. Various platforms with distinct capabilities are employed in proteomic studies to analyze samples [28]. These platforms include matrix assisted laser desorption ionization time of flight (MALDI-TOF), electrospray ionization liquid chromatography/mass spectrometry (ESI-LC/MS), FAB-MS (fast atom bombardment mass spectrometry), ion trap instruments and Fourier transform mass spectrometer (FT-MS) systems. The precision of these methods relies on accurately determining the peptides molecular mass charge ratio, as well as its chemical structure, using high resolution and sensitivity [5].

The peptides that were isolated are thoroughly identified and understood through the use of spectroscopic analysis, including 1D and 2D NMR techniques, alongside mass spectrometry [29]. NMR spectroscopy stands out as one of the most effective analytical tools available today for determining the structure of bioactive compounds. The principle behind NMR is based on nuclear spin, which means that when a molecule is placed in a magnetic field, its momentum aligns either in the same or opposite direction to the field. This alignment creates two distinct states separated by an energy gap and results in resonance frequency. The difference in resonance frequency depends on both the chemical environment of the nucleus within a molecule and the strength of the magnetic field [27].

1. **HEALTH-PROMOTING PROPERTIES OF MARINE-DERIVED BIOACTIVE PEPTIDE**

Marine derived bioactive peptides have beneficial qualities, including antioxidant properties, antimicrobial activity, the ability to regulate blood pressure, anti-diabetic and anti-obesity effects, anti-inflammatory effects and immune system modulation (Figure 2) [30].

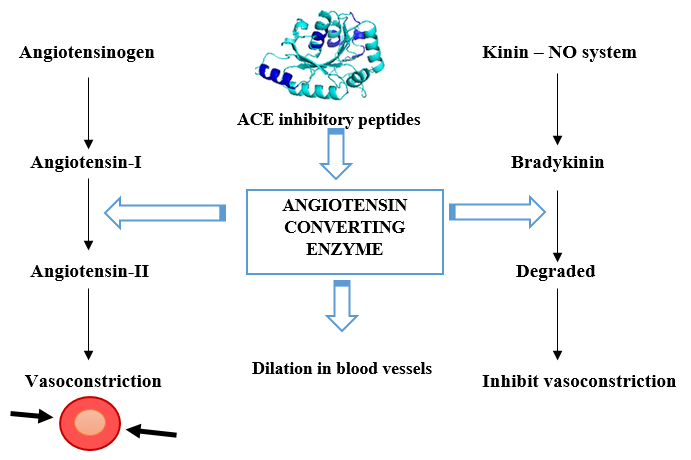
**Figure 2. Main health-promoting properties of bioactive peptides isolated from marine resources**

1. **Blood Pressure-Lowering Effect**

Cardiovascular diseases (CVDs) are a collection of conditions that impact the heart and blood vessels. They are often referred to as heart diseases or disorders related to the heart and circulatory system [31]. The World Health Organization (WHO) reports that CVDs are responsible, for the loss of 17.9 million lives [32]. High-blood pressure (hypertension) is the one of the main risk factor for CVDs. Hypertension drugs, treatments that inhibit the angiotensin I converting enzyme (ACE; EC 3.4.15.1) are commonly employed to manage blood pressure levels in the renin-angiotensin system. ACE is present in several organs such as the lungs, blood vessel walls, brain and kidney [33]. ACE, also known as dipeptidyl-carboxypeptidase, is an enzyme that breaks down peptides into dipeptide units by cleaving them at the C terminus. One of its significant roles is converting angiotensin-I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) into angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe), an active vasoconstrictor that elevates blood pressure. Additionally, ACE degrades and inactivates Bradykinin, which normally promotes arterial relaxation and lowers blood pressure. These processes collectively work together to directly elevate blood pressure (Figure 3). ACE inhibitors, as hypertension drugs, are employed to suppress these responses, causing vasodilation and ultimately lowering blood pressure [8]. Captopril, enalapril, lisinopril and benazepril are frequently prescribed as synthetic ACE inhibitors to effectively manage hypertension. On the hand it should be acknowledged that synthetic medications, such, as ACE inhibitors can sometimes bring about side effects. Some typical side effects associated with ACE inhibitors are feelings of dizziness, persistent coughing, increased levels of potassium, in the body and allergic reactions [34].

In recent times, there has been increasing interest in exploring natural marine products as potential alternatives to synthetic drugs. Due to their various beneficial effects, natural marine products have captured the attention of numerous researchers [33, 35, 36]. ACE-inhibitory compounds have been extracted from seaweed [37], fish gelatin [38], algae [39], rainbow trout [40], jawless vertebrate lamprey [41], and Atlantic sea cucumber [42]. Most of the reported peptides act as competitive inhibitors of ACE. In the mode of inhibition, the inhibitor engages in a competition with the substrate by binding to ACEs active site. On the other hand, in non-competitive inhibition mode the inhibitor attaches itself to a distinct location leading to a change in ACEs shape that obstructs the substrate, from binding to its active site. Consequently, complexes of enzyme-substrate and enzyme-inhibitor are formed, and they cannot interact simultaneously. The inhibitory peptide's C-terminal end interacts with ACE's active site pockets, which consist of three sub-sites: antepenultimate position (S1), penultimate position (S1’), and ultimate position (S2’). Among the substrate's amino acids, Pro, Ala, Val, and Leu are most favorable for S1; Ile is the most favorable for S1’, and Pro and Leu are the most favorable for S2’. The S1 sub-site comprises residues Ala354, Glu384, and Tyr523; the S1’ pocket contains Glu162; and the S2’ pocket includes Gln281, His353, His513, Lys511, and Tyr520 [33].

The effectiveness of peptides sourced from marine organisms is quantified through their half maximal inhibitory concentration (IC50), a measure that signifies the concentration of ACE inhibitor necessary to achieve a 50% reduction in ACE activity. Additionally, the inhibition mode of ACE inhibitory peptides is commonly assessed using Lineweaver–Burk plots. Numerous techniques have been reported for *in vitro* measurement of ACE activity, including spectrophotometric, fluorometric, radiochemical, high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) methods [43]. The widely used spectrophotometric assay for ACE activity measurement was initially developed by Cushman and Cheung [44]. In summary, this method involves the extraction of hippuric acid (HA), formed from the substrate hip–his–leu (HHL) due to ACE action, using ethyl acetate and then quantifying it through a spectrophotometric assay. Several modifications to this method have been reported, such as substituting the ethyl acetate extraction with specific binding of His–Leu using 2,4,6-trinitrobenzene sulphonate (TNBS) or a specific reaction of HA with benzene sulfonyl chloride [43]. While the spectrophotometric assay has proven to be valuable, it is characterized by its complexity, time-consuming nature, and limitations in analyzing trace amounts of samples. As an alternative, reverse phase HPLC has been employed to determine ACE activity by measuring the extracted HA content through a UV monitor [45]. Using an HPLC method with the C18 column, ACE-inhibitory activity can be directly determined without the need for HA extraction. This approach achieves complete baseline separation of HA from HHL with enhanced sensitivity [46].



**Figure 3. Basic mechanism of ACE inhibition effect of bioactive peptides**

Fresh tilapia *([Oreochromis niloticus](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/oreochromis-niloticus" \o "Learn more about Oreochromis niloticus from ScienceDirect's AI-generated Topic Pages)*) filleted, deskinned, and minced. Fish samples were defatted with isopropanol and lyophilized. The crude proteinases were added to the mixture at concentration of 5 U/g and hydrolysis was done 60 °C with different hydrolysis times (0, 2, 4, 8 h). After hydrolysis, ACE inhibitory activity analysis of hydrolysates was performed using an HPLC. As a result of the research, ACE inhibitory activity of the hydrolysate increased with hydrolysis time. Additionally, at 8h of hydrolysis the most potent ACE inhibiton activity with an IC50 of 0.54 mg/ml. Modifications in the amino acid composition lead to alterations in the biological activity of the peptides generated through hydrolysis. The fraction with a molecular weight (MW) of less than 5 kDa exhibited the highest ACE inhibitory activity, displaying a specific inhibitory activity of 626.71%/mg peptide. Conversely, the fraction with MW greater than 30 kDa demonstrated the lowest ACE inhibitory activity. The longer chain peptides are generally more challenging to bind with the active site of ACE, resulting in a decrease in inhibitory activity. This could partially account for the lower inhibitory activity observed in the fraction with MW > 30 kDa. Conversely, despite the lower peptide yield, the fraction with MW < 5 kDa exhibited a nearly 4-fold increase in specific inhibitory activity compared to the crude hydrolysate. As a result, sequential ultrafiltration emerged as an effective method for concentrating ACE inhibitory peptides in the hydrolysates [47].

Marine red alga *Gracilariopsis lemaneiformis* protein was hydrolyzed using trypsin, flavourzyme, papain and alkaline protease. Among them, the trypsin hydrolysate displayed the most potent angiotensin-I-converting enzyme (ACE) inhibitory activity and was then divided into three fractions based on molecular weight: >10 kDa, 3–10 kDa, and <3 kDa. The <3 kDa fraction exhibited the highest ACE inhibitory activity of 78.15 ± 1.56% (2.0 mg/mL) and was chosen for additional purification. An ACE inhibitory peptide was extracted from the <3 kDa fraction using Sephadex G-25, G-15 gel chromatography, and ÄKTA pure system. The molecular mass and amino acid sequence of the purified peptide were determined as Gln-Val-Glu-Tyr (QVEY; MW, 537.57 Da) using MALDI-TOF/TOF–MS and MALDI-TOF/TOF–MS/MS, respectively. The peptide exhibited an IC50 value of 474.36 μM (0.255 mg/mL) [36].

For the purpose of the obtaining high quality protein hydrolysates from *Tetradesmus obliquus*, a green microalgae, seven different protein extraction methods based on chemical and mechanical techniques were utilized. This methods were suspension in lysis buffer and sonication using a commercial sonic bath, milling in presence of glass beads, alkaline process, HCl-Bligh and Dyer method, Hexane/ethanol, methyl tert-butyl ether, osmotic shock. After pretreatment, the mixtures were hydrolyzed by Alcalase and purified with solid phase extraction on C18 cartridges. Purified fragments were analyzed for bioactivity assays and most active fragments were identified by RP Nano HPLC-MS/MS. In results, among other peptides fragments, GPDRPKFLGPF and WYGPDRPKFL had a percentage of ACE inhibitory activity of 80%. Usually, ACE inhibitory effect is often linked to the presence of hydrophobic and aromatic components at the beginning and end of the molecule. In both peptides we observed an amount of these particular components, which led to their impressive effectiveness, with IC50 values of 5.73 and 0.82 μmol L−1 respectively [48]. Fish discards and by-products from the North-West Spain fishing fleets (whole fish, skin and head) hydrolyzed enzymatically by using Alcalase and the hydrolysates were dried by lyophilization for 72 h. Bioactivity assessment has been done on the dried samples.

In results, all fish protein hydrolysates (FPH) with a concentration of 5 mg/mL displayed significant ACE inhibitory activity ranging from 61.20% to 85.95%. Notably, FPH derived from fish heads generally exhibited lower ACE inhibitory activity, ranging from 60.77% to 79.35% [49].

Rainbow trout viscera protein hydrolysate was obtained using Alcalase enzyme at 60°C, pH 8.5 for 6 h. The viscera and its hydrolysates underwent in vitro digestion including oral phase (salivary fluid), gastric phase (pepsin solution), and intestinal phase. After digestion, soluble fractions were analyzed via Fast Protein Liquid Chromatography using AKTA purifier and thirty-six fractions with a molecular weight (MW) less than 10 kDa from the digested samples were automatically collected. Viscera exhibited the highest IC50 value (P < 0.05), indicating the lowest ACE inhibitory activity. However, the IC50 value of digested viscera was significantly (p < 0.05) lower, approximately 53.7% less than that of viscera, suggesting that potential antihypertensive peptides could be formed during the gastrointestinal digestion of viscera. While no significant differences were observed between the IC50 values of non-digested (H) and digested hydrolysate (HD), it can be inferred that ACE inhibitory peptides in H may be resistant to SGID (Simulated Gastrointestinal Digestion) or that other peptides with comparable activity might have been generated during the digestion process [50].

In another research, rainbow trout samples was hydrolyzed via papain and bromelain (0.05% *w*/*w*) at 50 °C for 1 h and protein hydrolysate was purified with 3 kDa cut-off ultra-filtration membrane. After that, the peptide fragments were tested for its bioactive properties. ACE inhibitory activity was reported as 55.6 ± 0.2% at 1035 µg/mL concentration and an IC50 value of 0.81 ± 0.0016 mg/mL [40].

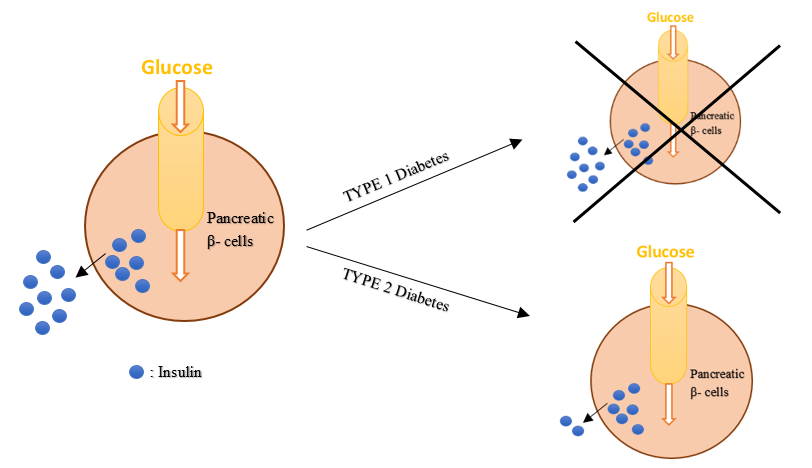
The renin-inhibitory tridecapeptide IRLIIVLMPILMA, derived from *Palmaria palmata* papain hydrolysate, exhibits its effects during gastrointestinal (GI) transit. After undergoing simulated gastrointestinal (GI) digestion, the degradation products were detected through mass spectrometry analysis, revealing the presence of the recognized renin and angiotensin I converting enzyme inhibitory dipeptide. Confirmation of the antihypertensive impacts of both the tridecapeptide IRLIIVLMPILMA and the seaweed protein hydrolysate, from which this peptide was extracted, was achieved through in vivo animal experiments employing spontaneously hypertensive rats (SHRs). After a 24 hour period, the group of spontaneously hypertensive rats (SHR) that received the protein hydrolysate from *P. Palmata* experienced a decrease of 34 mm Hg in their systolic blood pressure (SBP). Their SBP dropped from 187 (±0.25) mm Hg to 153 (±0.64) mm Hg. In comparison, the group that was given the tridecapeptide IRLIIVLMPLIMA showed a blood pressure decrease of 33 mm Hg. They went from an initial reading of 187 (±0.95) mm Hg to 154 (±0.94) mm Hg SBP at the beginning of the experiment [51].

For the production of bioactive peptides with potent angiotensin-I-converting enzyme (ACE) inhibitory (ACEi) attributes, Alcalase was chosen among five protease types to hydrolyze Skipjack tuna (*Katsuwonus pelamis*) muscle. Optimal hydrolysis conditions were determined via single-factor and response surface experiments. Utilizing alcalase under these optimized conditions using 2.3% enzyme dose, a temperature of 56.2°C and a pH of 9.4. The outcomes revealed a remarkably potent ACE inhibiting protein hydrolysate (TMPH) derived from skipjack tuna muscle, with the TMPH demonstrating an ACE inhibition activity of 72.71% at a concentration of 1.0 mg/mL. Subsequently, employing ultrafiltration and chromatography techniques to obtain six novel ACE inhibiting peptides from TMPH; Ser Pro (SP), Val Asp Arg Tyr Phe (VDRYF), Val His Gly Val Val (VHGVV), Tyr Glu (YE), Phe Glu Met (FEM) and Phe Trp Arg Val (FWRV). These peptides had molecular weights of 202.3 Da, 698.9 Da, 509.7 Da, 310.4 Da, 425.6 Da and 606.8 Da respectively. It is worth noting that SP and VDRYF demonstrated significant ACE inhibition activity with IC50 values of approximately 0.06 ±0.01 mg/mL and 0.28 ±0.03 mg/mL, respectively [52].

1. **Anti-Diabetic Activity**

Diabetes mellitus (DM) is a metabolic disease related to high level of blood sugar and it has become a notable issue, in public health worldwide. The global incidence of DM has seen an increase over the years. From 1980 to 2014 the number of individuals affected by this condition increased dramatically from 108 million to a 422 million. Low and middle income countries experienced a more rapid rise, in prevalence compared to high income nations. In 2019, DM and diabetes-related kidney problems lead to about 2 million deaths [53]. When blood sugar levels are high and the body can't effectively derive energy from the food we eat, it becomes problematic. The pancreas plays a vital role in regulating blood sugar levels, typically between 4-6 mM, by releasing insulin and glucagon [54].

DM is mainly categorized into two types and they can be listed as Type 2 diabetes (T2DM) and Type 1 diabetes (T1DM) nad gestational diabetes which effects females throughout pregnancy. Briefly, when the levels of glucose in the bloodstream increase, it signals the pancreatic β-cells to secrete insulin, allowing the body's cells to take in and make use of glucose. In T1DM, these cells are destroyed, resulting in a deficiency of insulin production. However, T2DM is marked by inadequate insulin production by the body or reduced responsiveness of cells to insulin referred to as insulin resistance (Figure 4) [55].



**Figure 4. Simplified scheme of the pathogenic influences on β-cells inT1DM and T2DM**

T1DM is a long-term condition that occurs when the immune system mistakenly attacks and destroys the beta cells in the pancreas. These beta cells play a role in producing insulin a hormone that helps regulate blood sugar levels by allowing glucose to enter the body’s cells for energy. When beta cells fail to secrete insulin it leads to an insufficiency of this hormone resulting in levels of glucose in the bloodstream (hyperglycemia). The exact cause of this immune system attack on beta cells is not fully understood. It is believed to be influenced by both genetic and environmental factors [56]. Type 1 diabetes is generally diagnosed during childhood or early adulthood [57]. Requires lifelong insulin therapy to effectively manage blood sugar levels. Individuals with T1DM must regularly control blood glucose levels and administer insulin through injections or an insulin pump. Individuals with T1DM should maintain physical activity and change their eating habits or manage their diets in order to maintain stable blood sugar levels. Since insulin plays a role in glucose metabolism individuals with T1DM can not naturally produce sufficient amounts of this hormone. Without treatment persistently high blood sugar levels can lead to significant health complications over time. Therefore it is crucial for those with T1DM to manage their condition in order to prevent potential complications like kidney damage, nerve problems, cardiovascular disease and vision impairments [58].

According to global data, around 415 million individuals are presently affected by T2DM. Projections suggest that without prompt interventions, this number could surge to 642 million by the year 2040 [59]. T2DM is a condition that affects the body’s metabolism and leads to high blood sugar levels. It occurs when the body either doesn't produce insulin or becomes resistant, to its effects. Insulin, a hormone produced by the pancreas plays a role in regulating blood sugar levels by allowing glucose to enter cells and provide energy. In T2DM the body’s cells don't respond properly to insulin leading to what’s known as insulin resistance. As a result the pancreas may try to compensate by producing insulin. However, over time it may struggle to keep up with the demand resulting in insulin production. Lifestyle factors like being overweight or sedentary and having a diet often contribute to T2DM. Additionally, genetic factors and family history can also play a role in its development. Unlike Type 1 which typically appears during childhood or early adulthood and requires insulin therapy from a stage, T2DM can sometimes be managed through lifestyle modifications. For the management of T2DM, there are various strategies are employed, including the regular use of different anti-diabetic medications, lifestyle changes, and regular physical exercises [60].

At present, there is no known medication that can fully cure T2DM. The current drugs mainly function by managing blood sugar levels enhancing the body’s response, to insulin and reducing the harm caused by hyperglycemia. Consequently, there exists a desire to discover or create more potent medications or treatments for tackling T2DM [61]. Bioactive peptides have the ability to improve the balance of glucose in the body and increase sensitivity to insulin [62, 63]. The researchers have shown significant interest in exploring new bioactive peptides, particularly from marine sources, due to the increasing population of individuals suffering from diabetes and the scarcity of effective anti-diabetic medications [64]. In recent years, anti-diabetic peptides have been obtained from various marine organisms, such as oyster [65], red seaweed (*Porphyra* spp.) [66], silver carp swim bladder [67], Atlantic salmon skin (*Salmo salar*) [68], Tilapia (Oreochromis niloticus) by-products [69], dogfish and shark [70]. The management of T2DM can be approached the inhibition of carbohydrate hydrolyzing enzymes, such as α-amylases and α-glucosidases, in the digestive organs [71,72]. Another method for treating T2DM is that lower glucose level, like inhibitors of dipeptidyl peptidase IV (DPP-IV) [73, 74, 75] (Figure 5). Because of their chemical structure, peptides have the capacity to interact with amino acids present in the catalytic site of the enzymes, thereby inhibiting their function [76].

**Figure 5. Bioactive peptides exhibit anti-diabetic effects for T2DM based on inhibition**

Salivary and pancreatic α-amylase inhibitors are known for their ability to inhibit the breakdown of starch into smaller sugar molecules (oligosaccharides), thereby preventing the absorption of starch glucose components by the human body. Additionally, inhibitors of α-glucosidase block the breakdown of di, tri and oligosaccharides into glucose within the small intestine [77]. By inhibiting both enzymes, the digestion rate of carbohydrates decreases, leading to reduced absorption and transportation of glucose into the circulation and combining the inhibition of α-amylase activity with α-glucosidase is considered an effective approach to managing diabetes [78].

Dipeptidyl peptidase IV (DPP-IV/CD26; EC.3.4.14.5) is a protein found in cell membranes that participates in a wide range of biological processes. This serine protease, consisting of 766 amino acids has the ability to break down dipeptides located at the beginning of proline or alanine residue. It is also known as CD26, a marker on the surface of lymphocytes or as a binding protein for adenosine deaminase (ADA). DPP-IV is widely distributed throughout the body in organs such as the pancreas, sweat glands, salivary and mammary glands, thymus, lymph nodes, intestines and biliary tract, kidney, liver, placenta, uterus, prostate, brain, blood cells and skin. It attaches itself to the plasma membrane. The enzyme DPP-IV plays roles in different physiological processes including degradation of incretion hormones like GLP-1 and GIP. The hormones GLP-1 and GIP which are commonly referred to as hormones are discharged into the bloodstream by specific cells, in the duodenum (known as K cells) and the mucosa of the intestine (known as L cells) correspondingly. This release occurs when food is consumed. These hormones called incretins help increase insulin secretion from the β-cells in the islets of Langerhans when we eat. They do this in a way that depends on the level of glucose. This is very important, for keeping our blood sugar levels balanced. After a meal the levels of GLP-1 and GIP hormones in the body increase by two to three times. These hormones collectively contribute to around 60% of the insulin released after eating. However, it is worth noting that both GIP and GLP 1 are broken down by an enzyme called DPP-IV since these hormones are naturally occurring substances in the gut. Unfortunately due to the action of the DPP IV enzyme, both GIP and GLP 1 have short durations (less, than 7 and 2 minutes respectively) [79].

A substance that inhibits DPP-IV boosts the effectiveness of both added and naturally occurring GLP-1 and GIP hormones by stopping their initial breakdown. Consequently, this leads to the prevention of their deactivation. In individuals who have T2DM, researchers have noticed a decrease in the body’s response to incretins. This results in insulin production higher levels of glucagon after meals and an increase, in glucose levels following meals. DPP-IV inhibitors increase the duration of action. Enhance the levels of active incretins in the bloodstream. As a result, when the levels of incretins rise it causes a decrease in glucagon production. This leads to an increase in insulin release a delay in emptying and a reduction, in blood sugar levels. As a result, DPP-IV inhibitors can enhance glucose tolerance by augmenting the incretin effect in patients with T2DM [73].

Vildagliptin, saxagliptin, sitagliptin and alogliptin have been approved as medications for diabetes treatment in both the United States and Europe. Although most artificial DPP-IV inhibitors are generally well tolerated there have been reports of potential side effects such, as nasal congestion, headaches and urinary tract infections [80].

The search for natural alternatives to pharmaceutical drugs has expanded to include the study of peptides derived from marine resources. Red seaweed laver (*Porphyra* species) samples are hydrolyzed by proteases (alcalase, neutrase, pepsin and trypsin) separately under their optimal conditions. The hydrolysis was carried out using an enzyme/substrate ratio of 1:100 (w/w) and a duration of 4 h. After hydrolysis, protein hydrolysates were separated using consecutive chromatographic techniques, and 10 fragments were obtained from RP-HPLC. Among these fragments, D2 inhibited α-amylase activity (88.67 ± 1.05 %) significantly at 1mg/ml protein concentration. In mass spectrometry analysis (ESI-Q-TOF-MS) 8 peptides in this fraction. In this analysis, were discovered two peptides; Gly- Gly-Ser-Lys and Glu-Leu-Ser. To confirm their potential, for inhibiting α-amylase, was synthesized these peptides chemically. The synthesized peptides showed activity with IC50 values of 2.58 ± 0.08 mM for Gly-Gly-Ser-Lys and 2.62 ± 0.05 mM, for Glu-Leu-Ser. The α-amylase inhibitory drug, sold commercially (acarbose) showed a much stronger effect in inhibiting α-amylase compared to the synthesized peptides when tested under the same reaction conditions. The IC50 value for acarbose was measured at 0.45 ± 0.01 mM, which was significantly lower (P < 0.05). The peptides that have been identified may not be as effective in inhibiting as acarbose, but this finding implies that the peptides obtained from pepsin digestion of laver protein could have potential benefits for health and could be used in functional foods and pharmaceuticals to manage diabetes. Acarbose, which is a sugar analog, works through competitive inhibition. However, the peptides analyzed in this study have different characteristics and would exhibit diverse inhibition behaviors [66].

Boarfish (*Capros aper*) protein hydrolysate was experienced at 50°C, pH 7.0 with Alcalase 2.4L and Flavourzyme 500. After 3.75 h incubation time, simulated gastrointestinal digestion of hydrolysate was done. DPP-IV inhibitor activity was tested on the digested hydrolysate. The hydrolysate underwent a process called semi-preparative reverse phase high-performance liquid chromatography. Fractions were collected between 6 and 12 minutes, 12-15 minutes, 15-21 minutes, 21-25 minutes, 25-29 minutes, 29-33 minutes and 33-45 minutes using a fraction collector. The RP HPLC fractions that showed the highest activity, namely F28 and F29, were subjected to additional separation using an ACQUITY UPLC system. Subsequently, these fractions were analyzed utilizing a micrOTOF Q II mass spectrometer. In result, the most potent DPP-IV inhibitory peptide (IPVDM) had an IC50 value of 21.72 ± 1.08 μM [59].

Salmon skin collagen was tested to simulate gastrointestinal digestion. After hydrolysis, the digests were lyophilized. DPP-IV inhibitor activity was measured on the digests and MS analysis were done. The findings showed that the effectiveness of collagen in inhibiting DPP-IV increased significantly (from 11.57 ± 1.84% to 53.63 ± 3.14%, P < 0.05) after undergoing simulated digestion in a laboratory setting. As a result, the cleavage sites of enzymes and the pretreatments applied to proteins can lead to the formation of peptides with varying sequences. While the amino acid in the second position of the peptide sequence is not Pro (P), having a Phe (F) at the C terminal of peptide sequences is a characteristic feature of DPP IV inhibitory peptides. Furthermore the presence of the amino acid Threonine (T), at the beginning and Leucine (L) in the position were also taken into account as contributors to the inhibition. As a result the two discovered peptides TKLPAVF and YLNF which were identified during this study could be regarded as peptides, with a level of DPP IV inhibition [81].

Sardinella (Sardinella sindensis) was hydrolyzed with using alcalase (pH 8, 50°C), pepsin (pH 2.5, 37°C), and papain (pH 6.3, 65°C). Enzyme: substrate ratio and hydrolysis time was set as %2.5 (w/w) and 3 h, respectively. As a source of compounds, 250 μg/mL of pistachio green hull extract (PGH) was added to the hydrolysates. After hydrolysis, ultrafiltration was experienced with 10 kDa and 2 kDa MW cut-off membrane for purification. After all, Peptide profiles were evaluated by HPLC and the fragments were assessed for α-glucosidase, α-amylase and DPP-IV inhibition assays. The papain and alcalase hydrolysates did not exhibit any effect. The pepsin hydrolysate showed a mild inhibitory effect. Upon fractionation, the best glucosidase inhibitory activities were observed in Alcalase 2–10. The interaction, between the extract and peptides had a negative effect. Based on the results it seems that this interaction may have reduced the availability of groups, in the inhibitor required for interacting with α-glucosidase. For the α-amylase inhibitory effects of the hydrolysates and fractions, the inhibitory effects of pepsin hydrolysate were found to be lower compared to papain and alcalase hydrolysates with the latter two exhibiting activities (p > 0.05). However, through the process of ultrafiltration the inhibitory effects of pepsin hydrolysate were increased. When it comes to papain hydrolysate fractionation did not have an impact, on α-amylase inhibition. On the other hand, fractionating the alcalase hydrolysate resulted in the alcalase10 fraction showing the inhibitory effect. The hydrolysate and fractions had an effect compared to acarbose (with an IC50 value of 29.28 mg/mL) which is the standard inhibitor. Overall the hydrolysates and fractions showed effects, against α amylase, rather than α glucosidase. In the DPP-IV activity assay, the hydrolysis process using papain resulted in a hydrolysate that displayed the effect. There was no difference observed between the effects of hydrolysates produced with pepsin and Alcalase. Upon fractionation, it was found that the low molecular weight fractions exhibited inhibitory effects compared to the high molecular weight fractions. Amongst the fractions, it was observed that fraction <2 of pepsin had an inhibitory effect. Despite having a proline content the Alcalase hydrolysate demonstrated an inhibitory effect, on DPP-IV compared to both the papain hydrolysate and pepsin 2-10 fraction. The pepsin <2 fraction, which had the proline level showed to be the inhibitor of DPP-IV. The activity of DPP- IV inhibitors in hydrolysates is influenced by both proline content and peptide size. Smaller fractions with proline content were able to inhibit DPP-IV enzyme activity. Both papain hydrolysate and pepsin 2-10 demonstrated proline content with no difference in their inhibition of DPP-IV enzyme activity. Diprotin A was used as a control. Exhibited an IC50 value of 4 μg/mL. Peptides that display strong inhibitory effects, on DPP-IV typically contain hydrophobic amino acids and/or proline within their sequence. Some bioactive peptides containing alanine and proline residues have been found to inhibit DPP-IV activity [82].

1. **Anti-Obesity Activity**

Obesity poses a multifaceted challenge to address. It is a significant and long-lasting health condition that can adversely impact various bodily systems. The prevalence of obesity is rapidly escalating worldwide. In years there has been an increase, in the number of people worldwide who are struggling with obesity, which poses a big challenge to public health [83]. According to the WHO, the prevalence of obesity has nearly tripled from 1975 to 2016. In 2016 approximately 1.9 billion adults were identified as being overweight and out of that number more than 650 million were classified as obese. In 2016 nearly four, out of ten adults, aged 18 and above were dealing with being overweight while around one, in eight individuals were classified as obese [84]. Being obese can heighten the chances of developing several health issues, such as diabetes, heart disease, osteoarthritis and specific forms of cancer [83].

Cellular accumulation arises from a disruption in the delicate equilibrium between the energy ingested and the energy expended. This leads to the development of obesity. A crucial enzyme known as lipase assumes a pivotal role in the intricate process of lipid metabolism within the human body. Lipase (EC 3.1.1.3) is an enzyme that specifically targets the ester bonds found in triglycerides. Its role is to break down these bonds, resulting in the release of free fatty acids, mono and di glyceride molecules. In the context of obesity management, certain inhibitors like "orlistat," a widely available medication, can slow down the absorption of free fatty acids into both the systemic circulation and adipocytes for obesity management [85]. Inhibiting this enzyme has proven to be a method for preventing obesity making it an important approach for evaluating obesity treatments, in laboratory settings [86]. Obesity at the cellular level is marked by a rise in the number of fat cells that develop from fibroblastic preadipocytes in adipose tissues [87]. Another approach to combat obesity involves restraining the growth, maturation and storage of preadipocytes or promoting the breakdown of stored fat [88].

Currently, the prevalent methods used to tackle overweight and obesity in today’s society include approaches such, as weight management programs, specific diets like calorie control and intermittent fasting devices for weight loss medications, for reducing weight, surgery and liposuction procedures. However, there are weight loss strategies, like surgery or liposuction that come with surgical risks, including the potential for serious complications and even death. While it is true that the combination of over-the-counter dietary supplements and temporary interventions, along with a consistent routine of healthy eating and physical activity, can assist in achieving weight loss, it is important to acknowledge that certain medications for weight loss have been withdrawn from the market due to negative side effects For instance, the use of aminorex and sibutramine has been linked to cases of heart problems, high blood pressure in the lungs and several other non-deadly cardiovascular events [89].

It is widely accepted that consuming diets in carbohydrates and/or fats along, with a lifestyle are the primary lifestyle factors associated with disrupted lipid metabolism. This disruption often leads to levels of triglycerides (TG) LDL cholesterol and imbalanced glucose regulation [90]. Investigating the capabilities of peptides derived from natural sources to hinder pancreatic lipase shows potential as a therapeutic approach for combating obesity. Pancreatic lipase inhibitory peptides have been derived from marine organisms such as crucian carp (*Carassius carassius*) [86], jellyfish [91], green and brown seaweed [92], and yellow catfish [93]. Fish muscle from Crucian carp (*Carassius carassius*) was optimized with the aid of response surface methodology. Fish muscle was hydrolyzed with neutral protease, alkaline protease, papain, and protamex (100 U/mL) for 14 h. The researchers carried out an evaluation to determine the ability of fish protein hydrolysate to inhibit pancreatic lipase. When using alkaline protease, the hydrolysates derived from fish water-soluble protein showed a higher rate of inhibition on porcine pancreas lipase compared to neutral protease, papain and protamex. The results showed that certain model terms were important, while the lack of fit terms did not have much significance. The optimal conditions for hydrolysis using alkaline protease were determined to be an initial pH of 11, a temperature of 39 °C, an enzyme dosage of 122 U/mL and a hydrolysis duration of 10 hours. It was observed that under these specific conditions, the activity of porcine pancreas lipase amounted to 53.04% ± 1.32% [86].

Peptides were produced from fresh Atlantic sea cucumber (*[Cucumaria](https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/cucumaria" \o "Learn more about Cucumaria from ScienceDirect's AI-generated Topic Pages)* frondosa) using the enzymes alcalase (A) and trypsin (T). The enzyme concentrations were set at 3000 U/g for Alcalase and 2000 U/g for trypsin, with a ratio of 1:3 (w/v). The mixture of reactions was left to incubate with gentle stirring at a temperature of 25 °C for a duration of 2 hours. The peptides were separated into different fractions using ultrafiltration membranes with varying cut-off sizes (10 kDa, 5 kDa, 3 kDa and 2 kDa). This separation process resulted in obtaining 14 distinct peptide fractions (A<10, A10 5, A<5, A5 3, A<3, A3 2, A<2, T<10, T10 5, T<5,T5 3,T<3,T3 2,T<2). After that, the focus shifted towards the peptides found in the fractions below 2kDa (A<2 and T<2) because of their strong inhibitory effects on multiple enzymes. To identify these peptides accurately and precisely analyze them using LC-MS/MS combined with Peaks software. The activity of peptides in inhibiting lipase was assessed by measuring the production of free fatty acids from olive oil at a temperature of 37 °C and a pH level of 7.5. In the results, the peptides strongly inhibited pancreatic lipase (5.3–17.0%), ACE (52.2–78.8%) and α-amylase (16.3–27.2%). Tripeptides with a significant presence of R residues were created and showed strong effectiveness in inhibiting lipase enzymes [94].

1. **Antioxidant Activity**

Oxidation is a process found in all living organisms, where the production of free radicals and other reactive oxygen species (ROS) plays a key role in signaling. However, an excess of radicals can contribute to various human diseases, including heart conditions, strokes, arteriosclerosis, diabetes and cancer. The heart, lungs and brain are particularly susceptible, to the effects of free radicals as they rely heavily on oxygen [95]. Antioxidants are substances that play a role in reducing the harmful effects caused by reactive species like reactive oxygen and nitrogen. In the context of food antioxidants are compounds that can slow down hinder or even prevent oxidation processes. However, there are worries regarding the risks and unfavorable opinions linked to synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These substances might have impacts on vital organs, like the liver, spleen, lungs and other internal organs in humans [25]. As a result, there has been a growing demand for natural antioxidants, in recent years. Natural antioxidants commonly employed in foods include vitamin C, vitamin E and polyphenols. In the case of vitamin C and vitamin E specifically, the antioxidant molecules are either recharged by accepting electrons, from types of antioxidants or they undergo recycling for tissue repair purposes [96].

Since Prokornýs report in 1991, where it was mentioned that specific hydrolysates of peptides and proteins can help reduce the speed of autoxidation and peroxide levels in fats a significant amount of research has been focused on investigating antioxidant peptides, with enhanced antioxidant properties [97]. The antioxidant properties of an antioxidant peptide are influenced by factors such as the composition of amino acids the sequence of acids in the peptides the weight of the peptide chain as well as the amino acid residues, at both ends (N terminal and C terminal) and their hydrophilic characteristics [95].

It has been reported that there is a link between the way amino acids in peptides are structured and their antioxidant activity. Specifically, when the second L-histidine, in Pro-His-His is replaced with D-histidine it significantly reduces the peptide's antioxidant activity. The relationship between the properties of peptides and their amino acid structures was found to be meaningful. In a research performed an experiment where they created peptides without Tyr, Tyr-Phe, Tyr-Phe-Tyr and Tyr-Phe Tyr-Pro. These peptides were derived from the antioxidant peptide called Tyr-Phe-Tyr-Glu-Leu, which was originally extracted from casein. Different sequences showed varying levels of activity; Glu-Leu (EL) demonstrated the activity, followed by Tyr-Phe-Tyr-Pro-Glu-Leu (YFYPEL) Phe-Tyr- Pro-Glu-Leu (FYPEL), Tyr-Pro-Glu-Leu (YPEL) and Pro-Glu-Leu (PEL). This indicates that the EL sequence is particularly important for its activity [98]. Furthermore, a relationship between weight and antioxidant activity has been established. Peptides that have low molecular weights have a tendency to move through biological membranes more easily reaching the desired location [99].

Marine-derived peptides have shown increased levels of antioxidant activities. In the years scientists have discovered multiple anti-oxidative peptides derived from marine organisms such as Antarctic Krill (*Euphausia superba*) [100], marine algae (*Gracilariopsis lemaneiformis*) [101], and marine by-products [102, 103, 104].

Two peptides possessing antioxidant properties were extracted from skin protein hydrolysates of horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*) using a series of fractionations, such as ion exchange chromatography and gel filtration chromatography. The peptide sequence in the skin protein hydrolysate of horse mackerel was determined to be Asn-His-Arg-Tyr-Asp-Arg (856 Da) through electron spray ionization mass spectrometry (ESI MS/MS) while that of croaker was identified as Gly-Asn-Arg- Gly-Phe-Ala-Cys-Arg-His-Ala (1101.5 Da). The antioxidant activity of these peptides was assessed using electron spin resonance (ESR) spectrometry with 1 diphenyl 2 picryl hydrazyl (DPPH·) and hydroxyl (OH·) scavenging assays. Both peptides demonstrated efficacy in inhibiting peroxidation of polyunsaturated fatty acids (PUFAs) compared to α tocopherol, a natural antioxidant. These findings indicate that the two isolated peptides, from horse mackerel and croaker skin protein hydrolysates possess antioxidative properties and hold promise as potential food additives or pharmaceutical agents [105].

The peptides Leu-His-Tyr, Leu-Ala-Arg-Leu-Gly-Gly-Glu, Gly-Ala-His-Gly-Ala-Trp-Ala, Pro-His-Tyr-Leu and Gly-Ala-Leu-Ala-Ala-His were derived from the waste produced during the processing of sardinelle (*Sardinella aurita*). These peptides were obtained by utilizing an enzyme extract, from sardine (Sardina pilchardus). Among these peptides, the first tripeptide showed the ability to scavenge DPPH radicals. It is likely that the presence of the amino acid sequence His-Tyr contributes significantly to the activity of these peptides [106].

1. **Anti-Microbial Activity**

The excessive and inappropriate use of antibiotics worldwide has caused an increase in microbial resistance [107]. This poses challenges to human health as we are now witnessing the re-emergence of infectious diseases and a rapid rise in the number of pathogenic bacteria that are resistant to commonly available antibiotics. This situation threatens to bring us to a time before antibiotics were discovered. To address this issue scientists are prioritizing the search for antibacterial molecules that can effectively combat resistance. One promising avenue of research is exploring occurring cationic antimicrobial peptides (AMPs) which have caught the attention of scientists as potential alternatives to conventional antibiotics. These AMPs have been found in organisms and play a crucial role in their defense mechanisms against infections. The number of AMPs that have proven effective against bacteria continues to grow. They are small peptides (<60 amino acids) with broad-spectrum activity against microorganisms such as gram-positive and gram-negative bacteria, fungi, viruses and parasites [108]. An additional advantage is their likelihood of developing resistance. Furthermore, AMPs may serve functions beyond their antibiotic properties. In reality, certain peptides possess properties that can combat cancer. They also have the ability to enhance the system through promoting the release of cytokines facilitating chemotaxis and antigen presentation initiating angiogenesis and inflammatory responses as well as inducing adaptive immune responses [109].

Currently, antimicrobial peptides (AMPs) are an area of research for potential drug candidates. This is because they have a range of activities and importantly they may be able to combat antimicrobial resistance. AMPs work by targeting the lipid components in the invading pathogens membranes, which disrupts this structure and creates a barrier against the development of resistance. Due to this fact scientific research has focused on exploring the world of AMPs with, over 2000 reported in antimicrobial peptide databases [110].

Despite the potential of AMPs, they do have some drawbacks. These include stability under certain pH conditions and the ability to disrupt the cellular membrane of eukaryotic organisms which can lead to hemolytic side effects high production costs and technical challenges in manufacturing them. Additionally, there is a lack of data regarding their toxicity, pharmacodynamics and pharmacokinetics properties. Furthermore, their activity can be reduced when exposed to cations, like calcium and iron or specific serum conditions [111]. Antimicrobial peptides can be categorized into classes; (i) those consisting of α-helices (ii) small proteins and β-sheet structures, (iii) peptides containing thio-ether rings, (iv) peptides, with an abundance of one or two specific amino acids (v) lipopeptides and (vi) macrocyclic cystine knot peptides. This classification is based on research findings [112].

Marine-derived antimicrobial peptides have been obtained from many resources such as fish species [113,114], black tiger shrimp [115], and marine invertebrates [112].

A peptide called XL Asp P1, which has properties was discovered in the skin of *Xenopus laevis*. It has shown to be highly effective, against both gram-positive and gram-negative bacteria, well as having promising inhibitory effects, on breast cancer cells. Researchers were able to understand how this unique peptide works by using transmission electron microscopy. The antimicrobial activity is attributed to its ability to damage the cell membrane [116].

1. **Other Health Effects**

Inflammation refers to the body’s response, to types of injury such as trauma, infection, lack of blood flow, toxins or autoimmune conditions. It involves an interaction between cells and substances in any part of our body. Typically inflammation plays a role in fighting infections and promoting wound healing. There are two types of inflammation; acute and chronic. Acute inflammation is the response against pathogens, injured tissue or small particles. It is a process that self-limits over time. Acute inflammation is mediated by substances like eicosanoids and vasoactive amines that promote the movement of plasma and immune cells to the area. However, if phagocytes fail to eliminate pathogens or the immune system cannot restore tissue during inflammation it can progress into chronic inflammation. Chronic inflammation involves the release of cytokines and growth factors that attract advanced immune cells like leukocytes, lymphocytes and fibroblasts. Unfortunately during inflammation, these cells can cause damage, to tissues. Numerous research studies have been conducted to investigate the connection, between inflammation and conditions such, as heart attacks, Alzheimer’s disease and cancer [117]. Despite the importance of inflammation, there have been efforts to develop therapies that specifically target the inflammatory aspect of cardiovascular and malignant diseases. One class of drugs known as anti-inflammatory drugs (NSAIDs) such as aspirin are widely utilized for preventing and managing cardiovascular diseases due to their antithrombotic and anti-inflammatory properties. Recent research indicates that NSAIDs might also have effects against cancers expanding their potential applications in anti-inflammatory treatments. However, the known side effects, like bleeding and ulceration limit the long-term usage of NSAIDs for a significant portion of the population [118].

The researchers have provided details, about the range of peptides found in environments including their ability to combat inflammation. They have also discussed the inflammatory properties of newly discovered compounds derived from sponges, bacteria and microalgae [35].

The impact of peptides, on inflammation has predominantly been studied in mammalian cells. In this context researchers have explored the potential of peptides derived from various marine resources [119]. According to a study, three specific peptides (LREMLSTMCTARGA, AVGPAGPRG and VAPAWGPWPKG) found in the hydrolysate of a sea cucumber species called A*. Lecanora* displayed inflammatory properties. The effectiveness of these peptides was measured at 76.3%, 66.6% and 69.9% respectively [120].

In a research, scientists examined how oyster peptides (OPs) can impact the system of the mucosa and intestinal micro flora in mice that have been made immunosuppressed, through treatment with chemotherapy called Cyclophosphamide (Cy). The mucosa immune system consists of lymphocytes, macrophages and plasma cells. Acts as the initial defense against potential harm from the environment. Cyclophosphamide has effects, on the mucosa [121].

Immunostimulating or immunomodulatory peptides consist of categories of peptides including anticancer peptides and anti-inflammatory peptides. Anticancer peptides can have effects, on cancer cells, such as promoting cell death inhibiting tumor growth and regulating the system. They typically have molecules and are primarily made up of neutral and aromatic amino acids (for example Arg- Arg-Trp-Gln-Trp-Arg). On the other hand, anti-inflammatory peptides work by targeting the body's defense mechanism against inflammation. They help regulate the secretion of inflammation-inducing substances like cytokines or tumor necrosis factor-alpha (TNF-α). Anti-inflammatory peptides are characterized by a content of both hydrophobic and polar acids (for instance Tyr-Leu) [122].

There are types of peptides that help reduce cholesterol levels in the body each, with its unique way of working. They have the potential to either interfere with enter hepatic circulation, causing a rise in the elimination of cholesterol and bile acids through feces, or to enhance the expression of lipoproteins (LDL), achieving the same outcomes. It is known that many of these peptides work by inhibiting an enzyme called 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, which ultimately leads to the release of cholesterol. These peptides primarily consist of acids such, as proline, valine and glycine (for example; Gly-Gly-Val) [46]. Nevertheless, in a study, it was found that the reduction in cholesterol brought about by peptides is primarily linked to their primary or higher-order structure, rather than their specific amino acid composition. In fact, hydrophobic peptides have the ability to bind with cholesterol and bile acids [123].

The human sense of taste can perceive four primary tastes; sweet, sour, bitter and salty. Additionally, umami is recognized as the fifth taste by our sense of taste. While initially not considered a biological characteristic of peptides, scientific evidence has demonstrated that peptides derived from marine products and their derivatives can exhibit all five tastes [124]. This discovery holds significant potential for the growth of the food industry. Researchers conducted a study to examine the creation of protein hydrolysates from the heads and backbones of salmon, mackerel and herring. Afterward, a group of highly trained sensory evaluators assessed the sensory characteristics of these hydrolysates. Protein hydrolysates made from herring were found to have the strongest flavor, while those made from salmon were considered more enjoyable. This study revealed that the type of raw material used (heads or backbones) and the enzyme employed had minimal impact on sensory and nutritional characteristics. Instead, it was the choice of fish species that played a significant role in determining the taste profile of the protein hydrolysates [125].

To summarize, we have previously discussed the main biological effects triggered by peptides. However, researchers have also explored other bioactivities apart from the ones mentioned. These include antithrombotic, mineral binding, opioid, anti-stress and insomnia relief effects [126]. This emphasizes that the effects triggered by peptides may depend on various factors such as their structure, amino acid composition and the protein family they originate from.

1. **RESULTS & FUTURE RESPECTS**

Marine bioactive peptides are functional components that can prevent and treat diseases in maintaining health. The increasing demand for functional foods increases the need for functional food components. Bioactive peptides Its positive effects on health increase consumer awareness, research and production trends of bioactive peptides. There is a great demand for in vivo studies to better understand the mechanisms of marine bioactive peptides and to promote their commercialization. However, their components are unknown. It is important to identify marine peptides and to investigate the mechanisms of their effects on health. It is expected that bioactive peptides will be commercially available in the near future. In this case, these peptides can be used both in the development of functional new products in the food industry and in pharmacy.

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